mice, like those generated by Koizumi et al. (2006), were viable and fertile and had preserved neocortical lamination, with no evidence of subcortical heterotopias. Similar to Koizumi et al.'s observations, mice mutant for both DCX and DCLK displayed profound disorganization in cortical layering and widespread axonal defects in the corpus callosum, anterior commissure, subcortical fiber tracts, and internal capsules. However, in contrast to Koizumi et al.'s results, less severe phenotypes were noticed in the corpus callosum, suggesting potentially divergent functions of DCLK, DCLK-DCX-like, CARP, and CPG16 isoforms in distinct axonal pathways. Interestingly, human neurons deficient in DCX displayed axon-growth defects similar to those seen in DCLK/DCX double-null mice. Disruption of DCLK and DCX function in neurons in vitro led to abnormal dendritic development, shorter axons and dendrites, and disrupted axonal transport of synaptic vesicle proteins during axon growth. Together, the studies by Koizumi et al. and Deuel et al. strongly suggest that DCLK and DCX play a synergistic role in neuronal migration and axon growth in embryonic cortex.

The diverse function of DCLK evinced from these three studies highlights the significance of dynamic rearrangement of microtubule cytoskeleton during corticogenesis and raises the intriguing question of how the functions of MAPs, like DCLK and DCX, are orchestrated during distinct stages of neuronal development in cerebral cortex. Clearly, understanding the complimentary as well as unique functions of DCLK and DCX during neurogenesis, neuronal migration, and neuronal differentiation will be critical. The differences in DCLK expression noticed in different domains of the developing cerebral wall in these three studies suggest that DCLK expression may change from a restricted expression within the ventricular zone during early stages of neurogenesis to an increasingly more restricted expression in postmitotic, migrating neurons and postmigratory, differentiating neurons as cerebral cortical development unfolds. It is attractive to hypothesize that this change in DCLK expression pattern during development may serve to coordinate neurogenesis with appropriate placement and connectivity of neurons. Considering the striking differences in phenotypes observed between DCX null mice and RNAi-mediated DCX knockdown, further analysis of neural precursor proliferation in DCLK, DCX mouse mutants described by Koizumi et al. and Deuel et al. will be informative. These mouse models will also be eminently useful in determining whether DCLK functions to regulate the neuronal fate of all dividing ventricular progenitors in a generic manner or whether it differentially regulates the fate of symmetrically and asymmetrically dividing neural precursors.

Though it is evident that DCLK and DCX can synergistically act during neuronal migration and differentiation, the nature of this interaction remains unclear. Is DCX a substrate for doublecortin-like kinase and what are the endogenous substrates for DCLK? Are the microtubule binding activities of DCX and DCLK differentially regulated? Do different DCLK isoforms have distinct patterns of developmental coexpression with DCX within neural progenitors and postmitotic cortical neurons? Does DCLK interact differentially with known DCX interactors such as AP-1, neurofascin, Lis1, MARK, cJNK, JIP, neurabin II, and Cdk5? Additionally, the suggestion that the DCX/DCLK pathway might regulate the vesicle trafficking (Deuel et al., 2006) needed to support membrane expansions during neuronal motility and process growth adds a new twist to DCX/DCLK's function in cerebral cortex. While these three studies provide novel and exciting perspectives on the role of DCLK in cerebral cortex, how doublecortin domain-containing proteins orchestrate their multiple functions during cortical development remains a challenging question.

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Cannabinoids in Microglia: A New Trick for Immune Surveillance and Neuroprotection

Microglia are the resident immune cells of the brain, and they are under permanent activity to patrol the

cerebral microenvironment. A proper inhibitory feedback onto these cells is critical during both intact and injury conditions. In this issue of *Neuron*, Eljaschewitsch and colleagues report that such feedback is provided by the endogenous cannabinoid anandamine and $CB_{1/2}$ receptor signaling, which ultimately leads to mitogen-activated protein kinase phosphatase-1 (MKP-1) induction. MKP-1 interferes with lipopolysaccharide-induced toll-like receptor 4 signaling and limits brain damage due to exaggerated microglial reactivity following acute NMDA injury.

There is a constant immune surveillance in the brain by microglia, which share similar characteristics as macrophages. Both macrophages and microglial cells derive from myeloid progenitors, but microglia are isolated in the brain parenchyma by the blood-brain barrier (BBB). Their direct interaction with pathogens is therefore quite limited, at least in intact brains. It is currently believed that parenchymal microglia originate from pial macrophages and mesenchymal progenitors from the yolk sac and that they establish themselves in the brain during the embryonic stage. However, recent data suggest the existence of two subpopulations of microglial cells, each of which may have different origins, i.e., the primitive macrophages from the yolk sac and those newly differentiated from monocytes. Over 95% of all microglia are born after birth and the formation of the BBB, and there has been an on-going debate regarding the maintenance of the microglial population in the adult CNS. One hypothesis is that adult microglia are maintained via self-replication or by the division of progenitor cells already present in the brain. Another hypothesis suggests that circulating monocytes are able to infiltrate the CNS and differentiate into microglial cells. Concrete evidence demonstrating the capacity of bone marrow stem cells (BMSCs) to populate the CNS and differentiate into microglial cells was recently obtained. The principal method to study BMSC infiltration into the brain is to transplant bone marrow cells from a donor animal into an irradiated host animal. With the use of this model, many researchers have found donor-derived cells in the brain of host animals, and BMSCs indeed have the ability to populate the CNS and differentiate into functional parenchymal microglia as well as perivascular microglia (Massengale et al., 2005; Simard and Rivest, 2004; Vallieres and Sawchenko, 2003).

The second debated concept regarding macroglia relates to their states of being either resting, alerted, or activated. Obviously, these states largely depend on the tools used to evaluate such an activity, and the shape of the cells was regularly used as a reference. The term "amoeboid cells" was previously used as an index of activation, but it is now believed to be a state of differentiation rather than activation. Indeed, amoeboid cells are monocytic cells in the process of being differentiated into ramified microglia. Recruitment of monocytes is particularly active in various models of CNS injury that are accompanied with BBB leakage. On the other hand, novel imaging technologies have markedly improved our knowledge on this matter. By using in vivo two-photon imaging in neocortex, Nimmerjahn and colleagues found that microglial cells are highly active in their presumed resting state, continually surveying their microenvironment with extremely motile processes and ramifications. An immediate and focal activation of microglia is also detected following BBB disruption (Nimmerjahn et al., 2005). Such a switch in their behavior from patrolling to shielding of the injured site is dependent on ATP/P2Y G protein-coupled receptor signaling mechanisms (Davalos et al., 2005). The high baseline motility of microglial processes may thus reflect the fluctuation of the ATP concentration in the surrounding tissue (Davalos et al., 2005).

The characterization of the toll-like receptor (TLR) family has also greatly contributed to a better understanding of the natural innate immune response by microglial cells. The Toll protein was first discovered as an essential molecule for the establishment of the dorsoventral axis in the *Drosophila* embryo. TLRs are mammalian homologs of this protein, which are expressed at the surface of a specific group of immune cells known as the antigen-presenting cells (APCs), in the brain microglia. These cells are rapidly activated by pathogens that bind to specific TLRs. A large family of TLRs, consisting of at least 12 highly homologous TLRs (10 in human and 12 in the mouse), has been characterized. The extracellular domains of all TLRs are comprised of 18 to 31 leucine-rich repeats and are very divergent from one another, indicating that these receptors recognize different molecules. The cytoplasmic domain of these proteins is highly similar to the cytoplasmic portion of the interleukin-1 receptor (IL-1R), and is therefore named the Toll/IL-1R (TIR) homologous region. It is now proposed that the various TLRs are key to the selective recognition of the major components produced by bacteria and viruses. These components are called pathogen-associated molecular patterns (PAMPs). PAMPs from Gram-positive bacteria are recognized by TLR2 conjugated to TLR6 or TLR1, double-stranded RNA (dsRNA) viruses are recognized by TLR3, LPS from Gram-negative bacteria binds to TLR4, flagellin to TLR5, and CpG bacterial and viral DNA triggers signaling via TLR9. Single-stranded RNAs (ssRNAs) from viruses (e.g., HIV-1 and influenza) are the physiological ligands for TLR7 and TLR8, whereas uropathogenic bacteria activate TLR11. It is also believed that TLR1, 2, 4, 5, 6, and 11 recognize extracellular bacterial components, while TLR3, 7, 8, and 9 are sensors for intracellular PAMPs in the phagosome (e.g., CpG DNA, ssRNA, and dsRNA). Human TLR10 has no mouse counterpart, and mouse TLR11–13 have no known ligands yet.

Because of the presence of the TIR domain, TLRs activate signaling pathways that are similar to those engaged by IL-1. The TIR domain can interact with MyD88 (Figure 1). This adaptor protein has an amino-terminal death domain (DD) that associates with the IL-1R-associated kinase (IRAK), a serine kinase that activates another adaptor molecule—tumor necrosis factor (TNF) receptor (TNFR)-associated factor 6 (TRAF6). Recruitment of TRAF6 leads to the activation of multiple kinases, which ultimately free NF-κB for triggering transcriptional activation of proinflammatory genes (TNF-α, IL-1β, IL-12p40,...). There are also MyD88-independent signaling events involving a different subset of accessory proteins, such as Toll/IL-1R domain-containing adaptor-inducing IFNβ (TRIF), TRIF-related adaptor molecule

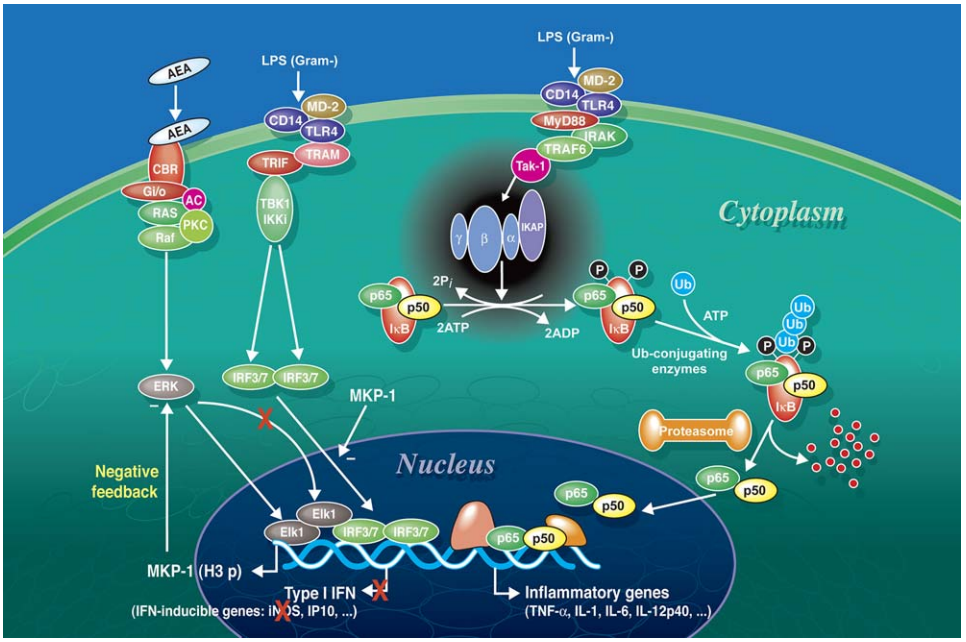

Figure 1. Schematic Illustration of the Hypothetical Interaction between Anandamine (AEA)/$CB_{1/2}$ Receptor Signaling and the MyD88-Independent Signal Transduction Pathway Engaged by the Binding of Lipopolysaccharide to Its Cognate Toll-Like Receptor 4

The high-affinity extracellular complex includes CD14 and MD-2. Such an interaction leads to the classical proinflammatory pathway involving MyD88, multiple kinases, and NF-κB, which triggers cytokine gene expression (e.g., TNF-α). TLR4 activation is also able to stimulate a different subset of cytokines (e.g., type I interferons) through a MyD88-independent pathway with the participation of the adaptor proteins TRAM, TRIF, and the transcription factors IRF3/7. Biosynthesis of costimulatory molecules (e.g., iNOS) largely depends on IRF3-induced IFNβ. The MyD88-independent signal transduction pathway may then be an indirect target of AEA, because Eljaschewitsch and colleagues found that $CB_{1/2}$ receptor signaling and MKP-1 synthesis (through histone H3 phosphorylation) were able to markedly suppress iNOS gene expression and NO production in microglia. Because LPS-induced TNF-α release remained essentially unchanged by modulating the cannabinoid system (data not shown), it can be concluded that the MyD88-dependent route of NF-κB activation is not involved in the inhibitory feedback of AEA on microglia.

(TRAM), and the transcription factors IRF3/7. The latter stimulate transcription of the type I interferons (IFNα and β), which then cause production of costimulatory molecules (iNOS, IP10, MHC class II,...).

Interaction between LPS and TLR4 has been widely studied in microglia both in vivo and in vitro. Systemic challenge with the endotoxin triggers transcriptional activation of inflammatory genes in microglial cells throughout the brain parenchyma. Such widespread microglial reactivity to LPS is dose and time dependent. Some genes are induced very rapidly (e.g., IκBα, TNF-α, IL-1β, CD14), while others take several hours to days to be detected (e.g., complement proteins). Direct injection of LPS into the CNS parenchyma also causes a strong and time-dependent transcriptional activation of inflammatory genes in microglial cells ipsilateral to the site of injection (Nadeau and Rivest, 2002). Such a robust and transient inflammatory response by microglia is not associated with neuronal damages or demyelination (Nguyen et al., 2002). Inhibition of these activated cells would seriously compromise the natural immune surveillance of the brain and prevent proper elimination of pathogenic substances in the cerebral microenvironment. If left unchecked, however, microglia cause damaging inflammatory responses reminiscent of neurodegenerative disorders (Nadeau and Rivest, 2003; Soulet and Rivest, 2003).

In this issue of *Neuron*, Eljaschewitsch and colleagues (Eljaschewitsch et al., 2006) report a new mechanism that provides an inhibitory feedback on microglia during both intact and degenerating conditions. They first show that absolute endogenous anandamine (AEA) concentrations increase in the brain of patients suffering of multiple sclerosis (MS), a disease with a clear immune etiology. Although the AEA levels were significantly higher in silent MS patients over the control, active MS patients had twice as many AEA concentrations than silent MS individuals. Coadministration of NMDA and the BV-2 microglial cell line also increased AEA levels in brain slice cultures, whereas exogenous AEA prevented microglia-induced neuronal damages in the presence of both NMDA and oxygen-glucose deprivation conditions. Of great interest here is the result that although the presence of BV-2 cells in the culture system was not by itself causing neuronal damages, these cells were needed for AEA-induced neuroprotection following NMDA insults. CB_2 was found to be a key receptor mediating these neuroprotective properties of the endocannabinoid system in microglia. Once again the presence of BV-2 cells in culture hippocampi did not lead to neurodegeneration, but CB_1 and CB_2 receptor antagonists were toxic to neurons. It can be concluded from these data that constitutive CB receptors restrict microglial activity to a proper immune surveillance and inhibiting the cannabinoid system would result in a loss of control of these immune cells in the CNS environment.

Such a mechanism fits very well with the ability of these cells to patrol the cerebral milieu where microglia are clearly beneficial to the neuronal elements. The binding of AEA to their cognate CB receptors is therefore one

of the mechanisms restricting microglia activity under basal conditions. This is also the case in inflamed conditions, because AEA was able to prevent LPS-induced nitric oxide (NO) production and iNOS gene expression in isolated BV-2 cells as well as coculture of brain tissue with BV-2 cells. It is important to note that AEA is unlikely to modulate the classical inflammatory reaction to LPS (Figure 1). IL-6 levels were not completely abolished, while AEA failed to change TNF-α production. It will be critical in future studies to investigate how CB receptor signaling interferes with TLR4 signal transduction pathways and NF-κB/IRF activity in microglia (see below).

In a series of very elegant studies, Eljaschewitsch and colleagues investigated the intracellular signaling pathways mediating the effects of AEA in BV-2 cells. They observed that the endogenous cannabinoids set microglia into a state of alert by a rapid MAPK phosphorylation and prevent overactivation in the presence of second stimulus. Indeed, MEK phosphorylation is reduced and $Erk_{1/2}$ dephosphorylation takes place after AEA incubation in LPS-activated BV-2 cells. This is associated with a rapid induction of the mitogen-activated protein kinase-phosphatase 1 and 2 (MKP-1 and MKP-2). Here, MKP expression was not necessarily a direct consequence of the MAPK activation; although AEA alone can activate the MAPK, it switched off this pathway by rapidly upregulating MKP-1 induction following exposure to LPS. The combined treatment of LPS and AEA (not LPS and AEA alone) enhanced histone H3 phosphorylation on the MKP-1 gene sequence. Finally, these authors show that AEA was able to inhibit BV-2 cells in a $CB_{1/2}$- and MKP-1-dependent manner and that MKP-1 can be found in microglial cells of MS patients. These data provide the first direct evidence that the CB receptors in microglia are coupled to Erk activation in regulating MKP-1 gene expression following histone H3 phosphorylation.

The authors reached the conclusion that the endocannabinoid AEA induces histone H3 phosphorylation, MKP-1 gene expression, and subsequent $Erk_{1/2}$ dephosphorylation in activated (e.g., LPS) but not in resting microglia, which in turn abolishes NO release and finally leads to neuroprotection. It is however important to keep in mind that all of these studies were performed in culture systems where brain slices were exposed to BV-2 cells and in one occasion to primary cultures of microglia. Direct interaction of these immune cells with CNS tissues taken from another group of animals may not represent the real situation taking place in vivo. Exogenous microglia may be under a different state of immune activation, which may be quite different from endogenous cells behind the BBB. This may modify TLR4 expression, the affinity to its ligand, LPS signaling, and expression of a subset of genes. Also, how these exogenous microglia interfere with their endogenous counterparts still remains to be determined.

The role of microglial cells in neurodegenerative disorders remains a matter of great controversy and debate at the moment. It is clear that LPS-induced proinflammatory signaling in microglia is a natural response that is unlikely to be detrimental to neurons and other cells of the CNS. In contrast, a proper immune response may set the conditions for swiftly eliminating pathogens in cases of cerebral infection, phagocyting cell debris after injuries, and improving brain repair. Recent data support this concept, because inhibition of microglia and TNF-α production was found to cause more damages following acute excitotoxicity (Turrin and Rivest, 2006), delay in remyelination, and inhibit recruitment of progenitors (Arnett et al., 2001). Genes encoding innate immune proteins are induced not only by PAMPs, but also in response to brain injuries and during a variety of neurodegenerative disorders. What comes first (inflammation or cellular degeneration) remains largely unknown, and the role of such an innate immune/inflammatory response in the cerebral tissue has yet to be fully unraveled. It is clear that sustained and unregulated inflammatory reactions are detrimental to neurons, though the acute release of proinflammatory molecules may instead play a leading role in protecting neurons against invading pathogens and restoring homeostasis after the storm. The direction that the inflammatory response is taking and the appropriate inhibitory feedback on microglia may consequently be crucial for determining the ultimate outcome of these events in the CNS.

As shown by Eljaschewitsch and colleagues in this issue, cannabinoid receptor signaling and MKP-1 gene expression in microglia may well be the ultimate trick for allowing these cells to either protect or contribute to neurodegeneration following acute brain damage. Future experiments are nevertheless needed to specifically define the physiological relevance of such a system in the mature as well as developing brain in an in vivo context. Moreover, how cannabinoid receptors interact with TLR4 signaling in microglia remains to be determined. It is possible that such an interaction does not involve the classical LPS-TLR4-NF-κB pathway, but the MyD88-independent signal transduction system. Although AEA had very clear effects on NO release and iNOS gene expression in BV-2 cells, cytokine levels were either not changed (e.g., TNF-α) or moderately modified (e.g., IL-6) in the presence of endogenous and exogenous cannabinoids. This supports the MyD88-independent set of events and the subsequent IFN release, which is a key step for iNOS gene expression and NO biosynthesis (Schilling et al., 2002). As depicted by Figure 1, this pathway may be the direct target of the so-called inhibitory feedback of the AEA/$CB_{1/2}$, Erk, and MKP-1 cascade. MKP-1 in causing Erk dephosphorylation and switching off MAPK would then lead to IFN gene repression. It is also tempting to propose a direct MKP-1/IRF interaction and dephosphorylation, such as in the case of $Erk_{1/2}$. These events together are powerful novel mechanisms to prevent the production of type I IFNs and their costimulatory molecules (e.g., iNOS) without interfering with the MyD88-dependent signal transduction pathway and cytokine gene expression. Although still speculative at this point, these data open the door for a potentially new treatment to inhibit specific signaling events with no side effects on those that might have neuroprotective properties in microglia. Of interest is the fact that exogenous cannabinoids (e.g., marijuana) seem to improve recovery, decrease frequency in relapsing, and delay demyelination in MS patients. These frequently discussed beneficial assets of marijuana have to be validated with in-depth studies, but MyD88-independent pathways may well be the indirect target of cannabis through $CB_{1/2}$ receptors and MKP-1 induction in macroglia and infiltrating macrophages. A new trick, yes indeed!

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Keeping Inhibition Timely

GABAergic interneurons play a key role in orchestrating cortical network oscillations. In this issue of *Neuron*, two studies (Bacci and Huguenard and Vida et al.) identify how networks of fast-spiking interneurons can enhance the regularity, precision, and robustness of their own rhythmicity via individual and collective self-innervation.

Neuronal networks of the mammalian cortex are comprised of two main classes of neurons: principal cells and GABAergic interneurons. Whereas principal cells excite other neurons to generate action potentials, the timing of those action potentials is to a large extent controlled by GABAergic interneurons. During active network states, characteristic oscillations in several frequency ranges emerge, at least partly due to the extensive feedback coupling between principal neurons and GABAergic interneurons. Some of the best studied of these network oscillations are the so-called gamma oscillations (30–100 Hz), which have been linked to cognitive processing (see Whittington and Traub, 2003). During gamma oscillations, fast-spiking (FS) GABAergic interneurons maintain a regular rhythm, tightly coupled to the population oscillation, whereas pyramidal neurons fire at a slower pace and are more loosely coupled to the on-going rhythm. How can the network of

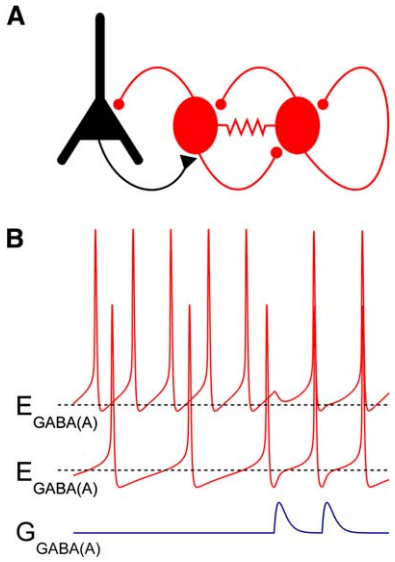

Figure 1. Diverse Mechanisms Underlying Interneuronal Synchrony

(A) Simplified wiring diagram showing autaptic and reciprocal connections between FS interneurons (red) and pyramidal cells (black).

(B) Homogenization and synchronization of firing by shunting inhibition in interneuronal networks. When the reversal potential for GABAergic events (E, dotted lines) is above the resting membrane potential, but below spike threshold, GABAergic conductances (G, lower trace) can decelerate strongly activated neurons (upper trace), while accelerating the firing in weakly activated neurons (middle trace). Computer simulation of Hodgkin-Huxley kinetics in a single-compartment model.

GABAergic interneurons remain in oscillatory synchrony in the presence of barrages of other on-going synaptic activity? By studying isolated GABAergic interneurons in brain slices and simulating their interactions, two new mechanisms have been identified that help achieve this (Bacci and Huguenard, 2006; Vida et al., 2006).

First, Bacci and Huguenard show that self-innervation, or so-called "autaptic" transmission, has the capacity to enhance spike fidelity in individual FS interneurons (Figure 1A). Anatomically, it is well established that FS interneurons innervate themselves (Tamas et al., 1997). These autaptic connections provide a brief GABA(A) receptor-mediated conductance following each action potential (Bacci et al., 2003). The function of this conductance has remained unclear. Now, Bacci and Huguenard link this conductance to network oscillations by showing an enhanced regularity and spike-timing precision mediated by these autaptic connections (Bacci and Huguenard, 2006). Blocking GABA(A) receptors dramatically increased spike jitter in interneurons, and the elegant use of dynamic clamp (Prinz et al., 2004) to mimic the autaptic connections was sufficient to restore the precision in spike timing. The use of dynamic clamp in this study was justified because autaptic connections target the somatic domain. Hence, adding an artificial conductance through the recording electrode at the soma can closely mimic the effect of synaptic input. There is a difficulty in extrapolating to the functional importance of these autapses in active networks, but Bacci and Huguenard also show that autaptic effects on both regularity and spike-timing precision are robust against synaptic noise, suggesting that the